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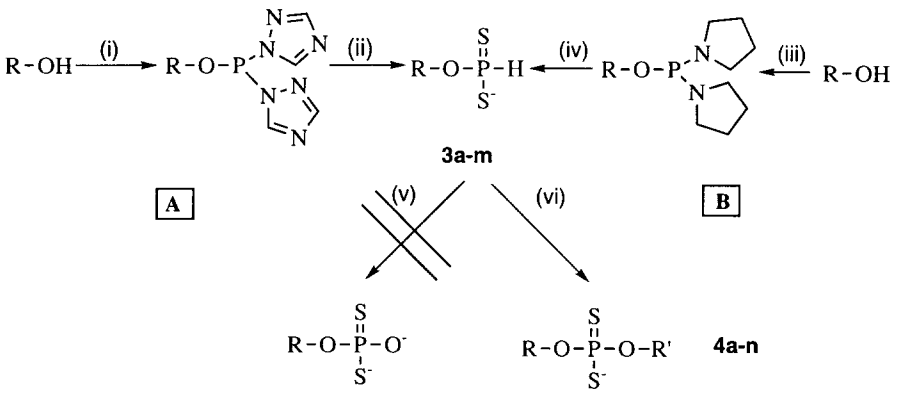
Oxidative Formation of Phosphorodithioates Via H-Phosphonodithioates

Peter H. Seeberger and Marvin H. Caruthers*

Department of Chemistry and Biochemistry, University of Colorado,
Boulder, CO, 80309-0215, USA

Abstract: H-Phosphonodithioate esters can be linked to a second alcohol via an oxidative coupling procedure to generate a phosphorodithioate diester. Nucleoside-cholesterol and nucleoside-amino acid phosphorodithioates were synthesized via this route.

Dithiophosphoric acid-O,O-diesters or phosphorodithioates have been used since the early 1960s as lubricants and fuel additives.[1] During the last five years, polydeoxynucleotide analogs containing dithioate linkages (dithioate DNA) have received increasing attention for biochemical and therapeutic applications as well.[2] This is because dithioate DNA is achiral, isoelectronic with respect to normal DNA, nuclease resistant, and a very potent inhibitor of Avian and Human Immunodeficiency Virus Reverse Transcriptases.[3-5] Several different methods for the synthesis of these analogs have been developed.[3-15]



Oxidative Formation of Phosphorodithioates. (i) PCl_3, N-methylmorpholine, 1,2,4-triazole; (ii) H_2S; (iii) tris-pyrrolidinophosphine; (iv) H_2S + tetrazole; (v) 1 eq 0.1 M iodine/pyridine + H_2O; (vi) 0.1 M iodine/pyridine + R'-OH.

An especially mild and versatile method for generating the phosphorodithioate internucleotide linkage is via the oxidative coupling of a 2′-deoxynucleoside 3′-H-phosphonodithioate with the 2′-deoxynucleoside 5′-hydroxyl group.[16] While this is not the method of choice for the synthesis of dithioate DNA, it is a useful procedure for preparing other biologically interesting phosphorodithioate analogs that cannot easily be prepared using the current methods.[3-15] Here we report our first attempts to synthesize some of these analogs via this oxidative approach – specifically cholesterol-nucleoside and amino acid-nucleoside hybrids.

Synthesis of H-phosphonodithioates. Two approaches leading to the synthesis of H-phosphonodithioates were examined. In method **A,** the tris-triazolophosphine was formed in situ from phosphorus trichloride and 1,2,4-triazole. 4-Methylmorpholine was used to neutalize the HCl produced during the reaction. After addition of an alcohol and H_2S, the H-phosphonodithioate was formed as the major product with the H-phosphonothioate as the main by-product (20-30%). Separation of these compounds by silica flash-chromatography yields the H-phosphonodithioates (**3a-3m**) in 38-65% as their triethylamine salts (Table 1). Method **B**, in which the alcohol was coupled to tris-pyrrolidinophosphine by activation with 1-H-tetrazole followed by hydrogen sulfenolysis, gave higher yields of **3a-3m** presumably because a more neutral salt was generated during condensation. For example, method B gave a much better yield of the H-phosphonodithioate derivative of the tyrosine *p*-nitrophenyl ester (Table 1). While some product formation with serine was observed by ^{31}P-NMR, we were unable to synthesize its H-phosphonodithioate in reasonable yields even with method B. As shown in Table 1, it is possible to generate several H-phosphonodithioates of primary, secondary and aryl alcohols in good yield and high purity via these two procedures.

Table 1. Yields and ^{31}P NMR Data of H-Phosphonodithioates

Compound	R-OH	^{31}P-NMR [ppm, CDCl3]	Yield [%]	Method
3a	5′-DMT-T	85.4	65.6	A
3b	5′-TBDMS-T	85.3	46.6	A
3c	5′-DMT-N6-Bz-dA	84.7	38.5	A
3d	3′-TBDMS-T	85.8	50.7	A
3e	3′-Ac-T	85.4	55.6	A
3f	3′-TBDMS-N6-Bz-dA	86.2	42.4	A
3g	3′-Ac-N6-Bz-dA	86.3	48.7	A
3h	Cholesterol	78.5	21.8	B
3i	Ac-Tyr-OEt	85.8	38.5	A
3j	Z-Tyr-OpNP	85.8	14.7	A
3k	Z-Tyr-OpNP	85.8	49.0	B
3l	Z-Thr-OMe	84.8	80.0	A
3m	Fmoc-Ser-OMe	88.2	14.5	B

Abbreviations. DMT: 4,4′-dimethoxytrityl; TBDMS, *tert*-butyldimethylsilyl; Bz, benzoyl; T, thymidine; dA, 2′deoxyadenosine; Ac, acetyl; Z, benzyloxycarbonyl; OpNP, para-nitrophenylester; Tyr, tyrosine; Thr, threonine; Ser, serine; OEt, ethylester; OMe, methylester; Fmoc, fluorenemethyloxycarbonyl.

Synthesis of Phosphorodithioate-Diesters. As reported earlier, the oxidative coupling of 2′-deoxynucleoside 3′-H-phosphonodithioate with the 5′-hydroxyl of 3′-acetylthymidine using iodine in anhydrous pyridine yielded dinucleoside phosphorodithioates.[16] Similar reactions of methanol, ethanol, and isopropanol with **3a** were followed by ^{31}P-NMR and yielded, in addition to the desired product, some material at 117 ppm (<5%) and some monothioate (50-60 ppm; <10%). The yield of desired product decreased in the order methanol>primary alcohol>secondary alcohol. The reaction proceeds rapidly and can be visualized by following decolorization of the iodine solution.

Table 2. Yields and ^{31}P NMR Data on Phosphorodithioates[a]

Compound	R-O-PS_2H	R′-OH	^{31}P-NMR [ppm, $CDCl_3$]	Yield [%]
4a	3a	Methanol	118.4	92†
4b	3a	Ethanol	115.5	83†
4c	3a	i-Propanol	113.0	80†
4d	3a	9-Hydroxyfluorenyl	116.7	53.3
4e	3a	20-(hydroxymethyl) pregna-1.4-dien-3-one	115.7	71†
4f	3a	9-Fluorenemethanol	116.2	51.4
4g	3d	9-Fluorenemethanol	113.3	59.6
4h	3e	9-Fluorenemethanol	113.4	44.0
4i	3c	9-Fluorenemethanol	112.3	40.4
4j	3f	9-Fluorenemethanol	112.7	22.5
4k	3g	9-Fluorenemethanol	113.5	36.8
4l	3a	Cholesterol	N.R.	-
4m	3h	3′-Ac-T	110.5	39.3
4n	3l	3′-TBDMS-T	114.3	52.3

[a]Yields calculated from 31P-NMR spectrum are indicated by a †, otherwise the reported results are isolated yields; N.R., no reaction.

Attempts to replace the alcohol with water in this reaction did not yield the desired product (the nucleoside dithiophosphate). Instead partially or fully desulfurized material was observed (^{31}P NMR) under several different sets of conditions. Perhaps this result was due to the formation of polyphosphates which hydrolyze to give desulfurized compounds. 2′-Deoxynucleoside dithiophosphates could, however, be synthesized from the 9-fluorenemethyl-phosphorodithioates **4f-k** by treatment with conc. ammonium hydroxide.[17] The steric environment of the alcohol appeared to have a large effect on the reaction. While it was possible to couple the cholesterol H-phosphonodithioate **3h** and the 5′-hydroxyl of thymidine, cholesterol would not react with 5′-DMT-thymidine 3′-H-phosphonodithioate **3a**. In the reactions in which sterically hindered alcohols were used, it proved useful to use 5-10 equivalents of the alcohol to drive the reaction to completion. Phenolic hydroxyl and silyl alcohols could not be used as R′-OH since they yielded mainly desulfurized products but no phosphorodithioates. Nucleoside amino acid conjugates containing a dithioate linkage were also synthesized via this same general procedure. This was demonstrated by the coupling of the 5′-hydroxyl of 3′-TBDMS-thymidine to the H-phosphonodithioate of threonine **3l**.

In conclusion, the oxidative formation of phosphorodithioates via H-phosphonodithioates constitutes a very versatile reaction for synthesizing phosphorodithioates having various combinations of nucleosides, amino acids, and complex alcohols such as cholesterol and 9-fluorenemethanol.

Synthesis of H-phosphonodithioates

General Method A. To a solution of 2.2ml (25 mmol) phosphorus trichloride in 250ml anhydrous dichloromethane, 5.75g (85 mmol) 1,2,4-triazole and 54 ml (0.25 mol) N-methylmorpholine were added and stirred for 30 min under argon. The mixture was cooled to 0 °C and a solution of 5 mmol of the alcohol in 75 ml anhydrous dichloromethane was added dropwise. After the addition was complete, the cooling was removed and the mixture was stirred at room temperature for 15 min. A steady stream of H_2S (dried over CaO) was bubbled through the solution for 10 min. After the reaction mixture was purged with argon for 30

min to remove excess H_2S, it was washed with 5% $NaHCO_3$ (2x 30 ml), the aqueous phase was re-extracted with 20 ml dichloromethane and the combined organic phases were dried over $MgSO_4$. The solvent was removed, and the crude product was purified by silica column chromatography (600g) (dichloromethane-ethylacetate-methanol-triethylamine, 60:30:5:5, v/v/v/v). The product containing fractions were combined and the solvent was removed in high vacuum.

General Method ***B***. To a solution of 5 mmol of the alcohol in 250 ml anhydrous dichloromethane, 1.15 ml (5 mmol) tris-pyrrolidinophosphine and 3.4 ml (1.7 mmol) of 0.5 M 1-H-tetrazole solution in anhydrous acetonitrile were added in 5 portions over 5 min and stirred for an additional 5 min. H_2S addition, workup, and purification were carried out as described for method **A.**

Synthesis of the Phosphorodithioates, General Procedure. A solution of 1 mmol of the H-phosphonodithioate **3a-l** in 10 ml anhydrous pyridine was added to a solution of one to ten equivalents of the alcohol. One equivalent of a 0.1 M solution of iodine in anhydrous pyridine was added dropwise until the dark color of iodine did not disappear. The mixture was stirred for 5 min and 1.0 M aqueous $Na_2S_2O_3$ solution was added to quench the excess iodine. The solvent was removed *in vacuo* and the crude product was purified by silica column flash chromatography (500g). Excess alcohol was recovered by flushing the column with 1 L of hexane-ethylacetate (1:1, v/v) and the pure product was eluted by 2 L dichloromethane-methanol (90:10, v/v). The product containing fractions were combined and yielded the triethylamine salts of **4a-n** after the solvent was removed *in vacuo*.

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